POSTER 5

PURIFICATION OF PRIMARY CELL WALLS FROM CORN ROOTS: INHIBITION OF CELL WALL-ASSOCIATED ENZYMES WITH INDOLIZIDINE ALKALOIDS

G. NAGAHASHI, P.M. BARNETT, S.-I. TU, AND J BROUILLETTE

INTRODUCTION

Recently, certain naturally occurring secondary plant products (indolizidine alkaloids) have been shown to inhibit α- and β-glycosidases isolated from plant (2,8,9,10) and animal cells (8,10). β-glucosidases (8,9) are inhibited by castanospermine (1, 6, 7, 8-tetrahydroxyoctahydroindolizine) and soluble, lysosomal, and Golgi associated α-mannosidases (2,10) are inhibited by swainsonine (1, 2, 8-trihydroxyoctahydroindolizine). These inhibitors have not been tested on plant cell wall-associated enzymes. In this report, a preparative cell wall purification procedure was developed in order to study the effects of castanospermine and swainsonine on cell wall-associated β-glucosidase and α-mannosidase activity.

MATERIALS AND METHODS

Corn seeds were germinated as described previously (5). Intact seedlings were submerged for 1 hr in cold distilled water to facilitate the removal of the cortex. The cortex was stripped from the stele (4) and primary cell walls were isolated and purified as shown in Fig. 1. Light and

appropriate for carrying out extensive enzyme kinetic analyses *in situ*. For this study, a preparative cell wall purification procedure was developed which used the Parr Nitrogen bomb as the major cell disruption step (Fig. 1). After purification, the large fragments of cortical tissue, as seen with the light microscope, did not contain any intact cells. At the ultrastructural level, no membranes, organelles, or cytoplasmic fragments were seen in the purified cell wall preparation. The lack of morphologically identifiable contaminants was confirmed by the lack of biochemical marker enzyme activity for membranes (cytochrome c oxidase, NADH cytochrome c reductase) and soluble enzyme (malate dehydrogenase, triose phosphate isomerase).

Because a recent report (6) indicated that cell wall associated enzymes may be basic in nature (pI > 7.0), an attempt was made to determine whether an artifactually bound protein (cyt c, pI = 10.6) was bound as tightly to the cell wall as were endogenous cell wall enzymes. Purified cell walls were preincubated with cytochrome c (10 mg ml^{-1}), washed several times to remove unbound cyt c , and then dissociation curves with increasing concentrations of NaCl or LiCl were determined. The dissociation curve of cyt c was compared to that of cell wall-bound β-glucosidase and results clearly showed that the endogenous enzyme was bound more tightly than the artifactually bound protein. At 0.1 M NaCl or LiCl, approximately 90% of the exogenous cyt c was dissociated while no β-glucosidase was released. Conceivably, a light salt wash of purified cell walls should readily remove any basic proteins which artifactually bound to the negatively charged cell wall during the cell disruption process.

Inhibition of Cell Wall Enzymes *In Situ*. Cell wall-associated β-glucosidase was inhibited by castanospermine and preincubation of cell walls with the alkaloid before enzyme assay had no effect on the degree of enzyme inhibition. Lineweaver-Burk analyses indicated that the cell wall β-glucosidase was competitively inhibited by castanospermine. The K_m for the uninhibited enzyme was 1.15 mM and the K_i was calculated to be 2.48 μM at pH 6.0. This K_i was smaller than that reported for a non-cell wall-associated β-glucosidase (8).

Unlike the β-glucosidase inihibition, the cell wall-associated α-mannosidase was further inhibited with increased preincubation time especially at lower concentrations of swainsonine. The preincubation effect by swainsonine has been reported for cytoplasmic associated α-mannosidases (2,10). Kinetic analyses were performed with and without preincubation conditions. With no preincubation treatment, the Lineweaver-Burk plot was curvilinear although at high substrate (1-5mM) concentrations the inhibition appeared to be competitive (K_m = 1.25 mM, K_i = 32 nM). Jack bean α-mannosidase was also competitively inhibited by swainsonine but the inhibition was very difficult to reverse (2,10). When cell walls were preincubated for 15 min with swainsonine before enzymatic assay, the Lineweaver-Burk plot again was curved. Although the inhibition by swainsonine appears to be complex, the data suggest that

α-mannosidase has two separate binding sites for this alkaloid.

***In Vivo* Inhibition with Indolizidine Alkaloids.** When intact roots were grown in 20 μg ml^{-1} of castanospermine for 2 days, three distinct differences were apparent when compared to the control. First, the root length was one half of the control. Second, the root tip including the zone of elongation was curled like a corkscrew. Third, no secondary roots were formed in the presence of the inhibitor. These results suggest that the cell wall β-glucosidase, *in vivo*, plays a role in cell wall metabolism (turnover and elongation).

In contrast, swainsonine (20 mg ml^{-1}) had no observable effect on growth. This indicates that the cell wall-associated α-mannosidase does not play a central role in cell wall metabolism. The α-mannosidase may have a role in host-pathogen interactions particularly as a part of the host defense system since fungi may contain various extracellular mannans (1).

The isolation of specific inhibitors of cell wall enzymes would provide a powerful tool for probing the functions of these biologically active cell wall proteins as well as providing a method for studying cell wall structure by selective inhibition during autolysis (3).

Acknowledgements--We thank Beverly Maleeff and Timothy Dobson for the light and electron microscopy and Dr. Russell Molyneux for the kind donation of indolizidine alkaloids.

LITERATURE CITED

1. CHIURA H, M IZUKA, T YAMAMOTO 1982 A glucomannan as an extracellular product of *Candida utilis* II. Structure of a glucomannan: Characterization of oligosaccharides obtained by partial hydrolysis. Agric Biol Chem 46: 1733-1742
2. KANG MS, AD ELBEIN 1983 Mechanism of inhibition of Jack Bean α-mannosidase by swainsonine. Plant Physiol 71: 551-554
3. LABRADOR E, G NICOLAS 1984 Autolysis of cell walls in pea epicotyls during growth. Enzymatic activities involved. Physiol Plant 64: 541-546
4. LEONARD RT, G NAGAHASHI, WW THOMSON 1975 Effect of lanthanum on ion absorption in corn roots. Plant Physiol 55: 542-546
5. NAGAHASHI G, AF BAKER 1984 β-glucosidase activity in corn roots: Problems in subcellular fractionation. Plant Physiol 76: 861-864
6. NAGAHASHI G, TS SEIBLES 1986 Purification of plant cell walls: Isoelectric focusing of $CaCl_2$ extracted enzymes. Protoplasma (in press)
7. NAGAHASHI G, TS SEIBLES, SB JONES, J RAO 1985 Purification of cell wall fragments by sucrose density gradient centrifugation. Protoplasma 129: 36-43

8. SAUL R, JP CHAMBERS, RJ MOLYNEUX, AD ELBEIN 1983 Castanospermine, a tetrahydrozylated alkaloid that inhibits β-glucosidase and β-glucocerebrosidase. Arch Biochem Biophys 221: 593-597
9. SAUL T, TJ MOLYNEUX, AD ELBEIN 1984 Studies on the mechanism of castonospermine inhibition of α- and β-glucosidases. Arch Biochem Biophys 230: 668-675
10. TULSIANI DRP, HP BROQUIST, O TOUSTER 1985 Marked differences in the swainsonine inhibition of rat liver lysosomal α-D-mannosidase, rat liver Golgi mannosidase II, and Jack Bean α-D-mannosidase. Arch Biochem Biophys 236: 427-434